乙型肝炎病毒染色液(Shikata 地衣红法)

货号：G1921
规格：3×50mL
保存：2-8℃, 避光保存, 有效期 6 个月。

产品组成：

名称		3×50mL	保存
试剂(A): Shikata 酸化液	A1: Shikata 氧化剂	25mL	2-8℃,避光
	A2: Shikata 酸溶液	25mL	室温
临用前，取 A1、A2 等量混合，即为 Shikata 酸化液，即配即用。			
试剂(B): Shikata 漂白液		50mL	室温
试剂(C): 酸性地衣红染色液		50mL	室温，避光

产品介绍：

病毒性肝炎是由肝炎病毒引起的肝实质细胞变性坏死为主要病变的传染病。乙型肝炎病毒是世界范围内传播的乙肝病毒，HBsAg 存在于肝细胞质内，目前可通过免疫组化方法显示乙型肝炎病毒的 HBsAg 和 HBcAg。显示 HBsAg 的方法有 Shikata 地衣红法、醛品红法、维多利亚蓝法等。

乙型肝炎病毒染色液(Shikata 地衣红法)操作简单、特异性高，但染色效果可能受到不同批次原料而有差异，其可能机制是地衣红与 HBsAg 内胱氨酸的双硫键结合，进而显色。

操作步骤：(*仅供参考*)

1. 组织固定于 10%的福尔马林，常规脱水包埋。
2. 切片厚 4μm，常规脱蜡至水。
3. 入 Shikata 酸化液氧化 5min。稍水洗。
4. 入 Shikata 漂白液漂白 2min，脱去酸化液的着色。流水冲洗 2min，蒸馏水稍洗。
5. 入 70%乙醇稍浸洗。
6. 入酸性地衣红染色液加盖浸染 5h 或更长时间。95%酒精分化至基底呈淡棕色为止。
7. 无水酒精迅速脱水，二甲苯透明，中性树胶封固。

染色结果：

HBsAg 阳性物质	棕红色至深棕色
背景	不同程度的淡红色

注意事项：

1. Shikata 酸化液必须即配即用，混合后久置会降低其氧化力。
2. 酸性地衣红染色液临用前应恢复至室温，染液可重复使用数次。
3. 染色 5h 后，可取出切片在 70%乙醇中浸洗一下，再入蒸馏水水洗，镜下观察。如染色较淡，可在 70%乙醇中浸洗后入酸性地衣红染色液染色，必要时可过夜染。
4. 地衣红染色时，脂褐素亦可称阳性反应。其区别方法是：脂褐素着色较深，在细胞质内呈大小均匀的小颗粒；HBsAg 着色较浅，呈细微颗粒状。
5. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Hepatitis B Virus Stain Kit(Shikata's Method)

Cat: G1921
Size: 3×50mL
Storage:2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent		3×50mL	Storage
Reagent(A):Shikata Acidizing Solution	A1: Shikata Oxidant	25mL	2-8℃, avoid light
	A2:Shikata Acid Solution	25mL	RT
Before use, mix A1 with A2 in equal amount to form Shikata Acidizing Solution. It is ready to use.			
Reagent(B): Shikata Bleaching Solution		50mL	RT
Reagent(C): Acidic Orcein Stain Solution		50mL	RT, avoid light

Introduction

Viral hepatitis is a kind of infectious disease, the major lesion is hepatitis virus and the degeneration and necrosis of liver parenchyma cells caused by hepatitis virus. Hepatitis B virus is the worldwide spread of hepatitis B virus. HBsAg exists in the cytoplasm of liver. At present, HBsAg and HBcAg of hepatitis B virus can be displayed by immunohistochemistry. The methods to display HBsAg include Shikata Orcein Stain Method, Aldehyde Fuchsin Method, Victoria Blue Method, etc.

This kit is simple and specific, but its dyeing effect may vary with different batches of raw materials. The possible mechanism is that orcein combines with the disulfide bond of cystine in HBsAg to display color.

Protocol(*for reference only*)

1. Fix the tissue in 10% formalin. Conventionally dehydrate and embed.
2. Cut the section into 4μm. Conventionally dewax and rehydrate.
3. Oxidize by Shikata Oxidant for 5mins.Rinse with water slightly.
4. Bleach by Shikata Bleaching Solution for 2mins to remove the color of Shikata Acidizing Solution.
5. Rinse with distilled water slightly.Immerge with 70% ethanol slightly.
6. Stain with Acidic Orcein Stain Solution under cover for 5 h or more.
7. Differentiate by 95% ethanol until show a clear light brown base.
8. Dehydrate by absolute ethanol rapidly, transparent by xylene , seal with resinene.

Result

HBsAg Positive Substance	Brown Red to deep Brown
Background	Light Red in different level

Note

1. Shikata Acidizing Solution must be used immediately, otherwise its oxidation power will be reduced.
2. Acidic Orcein Stain Solution should restore to RT before use, and it can be reused several times.
3. After dyeing for 5 hs, take out the section and wash it in 70% ethanol, then wash it in distilled water, and view under the microscope. If the dyeing is light, it can be soaked in 70% ethanol and then dye with Acidic Orcein Stain Solution. If necessary, it can dye overnight.
4. When dyeing with orcein, lipofuscin has a positive reaction. The difference is that lipofuscin has a deep color, presenting small particles of uniform size in the cytoplasm; HBsAg has a shallow color, presenting fine particles.
5. For your safety and health, please wear experimental clothes and disposable gloves.

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